Chapter 3

The Adipokines in Domestic Animal Reproduction: Expression and Role in the Regulation of Ovarian Function

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Abstract

Currently, it is clear that female reproduction is regulated by the hypothalamic–pituitary-ovary axis, which produces many hormones that control reproductive stages. It is therefore important to have knowledge of new regulators/hormones controlling reproduction in domestic animals. In female animals, energy metabolism and fertility are tightly connected, and reciprocally regulated. The adipose tissue is well known to be implicated in the secretion of several hormones, such as the adiponectin, resistin, chemerin, visfatin, vaspin and apelin, the so-called adipokines or "adipose tissue-derived hormones". Many reports indicate that adipokines regulate the ovarian follicles' development, the onset of puberty and/or ovulation. This chapter summarizes that several adipokines are expressed in the ovary and that they can regulate ovarian physiology such as the steroid hormone production, cell proliferation, apoptosis and oocyte maturation in different domestic animals like pigs, cows, goats, ewes, chickens and turkeys.

Keywords: ovary, expression, steroidogenesis, proliferation, adipokines, domestic animals

1. Introduction

The reproductive system in female domestic animals is regulated precisely by an intricate interplay of hormones produced by the hypothalamus, anterior pituitary and the ovaries. The interplay of hormones results in ovarian cyclicity in females, which in consequence leads to fertilization, delivery by the maintenance of pregnancy of offspring. Moreover, it is now clear

that fertility depends on the energy metabolism status. For example, in cattle, genetic selection for high milk production is associated to high negative energy balance in the post-partum period and reduced fertility [1]. In pigs, a negative energy balance and a decrease in body fat results in a reduction in litter size and viability of piglets [2]. In sheep, it is well known that an increase in availability of energy substrates is associated with an increase in prolificacy [3]. Furthermore, obesity and some metabolic disorders influence the reproductive hormones in women [4].

The adipose tissue is well known to be implicated in the secretion of several hormones such as adiponectin, apelin, chemerin, resistin, vaspin and visfatin: the so-called adipokines, which regulate energy balance, food intake, immunology and diabetes. A recent study indicated that several adipokines are expressed in the ovarian cells and that they can modulate ovarian physiology in some domestic animals, like pigs, cows, goats, ewes, chickens and turkeys [5].

2. Short description of adipokines

Adiponectin, apelin, chemerin, resistin, vaspin and visfatin were described as "adipose tissue-derived hormones". However, their expression and receptors are present in different tissues like the brain, stomach, kidneys, pancreas, liver or blood vessels. They play important roles in several metabolic processes, such as in the regulation of insulin sensitivity, food intake, adipogenesis and inflammation (**Figure 1**).

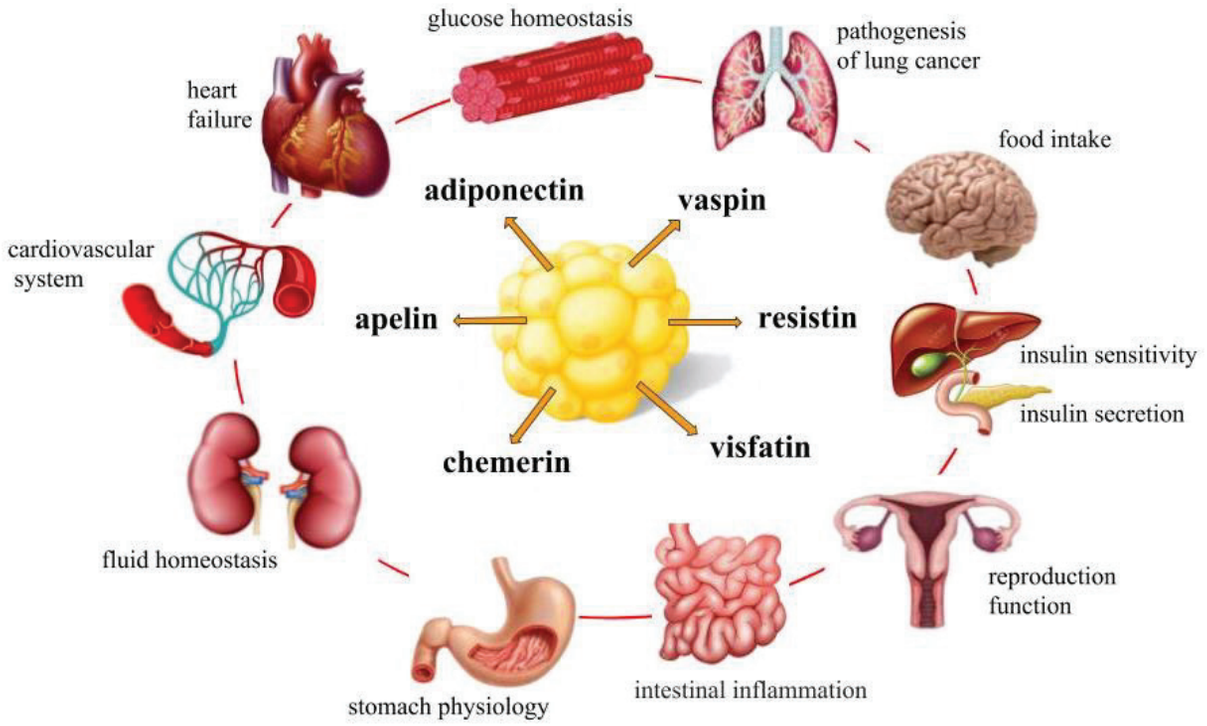

Figure 1. Adipokines and their receptors are present in different tissues like the brain, stomach, kidneys, pancreas, liver or blood vessels, and play important roles in several metabolic processes.

2.1. Adiponectin

Adiponectin is mainly produced by a white adipose tissue (WAT) and secreted into the bloodstream. The adiponectin level in serum is inversely related to body weight. Circulating levels range between 2 and 30 μg/ml in human plasma and are higher in females than in males [6]. In chicken plasma, the adiponectin levels are in the range of 4–10 μg/ml. The protein (26 kDa) was described for the first time by Scherer et al. [7] and is present in cells and plasma in three forms: trimers, hexamers and high-molecular weight (HMW) [8]. A number of post-translational modifications are required to obtain these forms. Three adiponectin receptors have been identified: AdipoR1, AdipoR2 and T-cadherin. The first two are the main adiponectin receptors and consist of seven transmembrane domains. AdipoR1 is abundantly expressed in skeletal muscles and is associated with the activation of AMP-activated kinase pathways. AdipoR2 is mainly expressed in the liver and is associated with the activation of peroxisome proliferator-activated receptor (PPAR)-α pathways. Then, T-cadherin is a receptor for hexameric and HMW adiponectin and is expressed on vascular cells and smooth muscles [9]. Adiponectin is an insulin-sensitizing, vascular protective, anti-apoptotic, anti-lipotoxic and anti-inflammatory protein on different cell types. Thus, it has been considered as a beneficial adipokine. Moreover, these functions mark this protein as a potent drug targeting diabetes and obesity-associated diseases [10].

2.2. Apelin

Apelin has been isolated from the bovine stomach extracts as an endogenous ligand of the previous orphan receptor APJ [11]. APLN gene encoding human apelin is located on chromosome Xq 25–26 [11]. Preproapelin, a precursor of the mature form, consists of 77 amino acids and is transformed into active forms by enzymatic hydrolysis. They are distinguished within four forms having different biological activities: apelin-36 (preproapelin 42–7), apelin-17 (preproapelin 61–77), apelin-13 (preproapelin 65–77) and pyroglutamate-apelin-13 (Pyr-apelin 13) [11]. The shorter apelin-13 has much higher biological potency than the longer apelin-36. Mature apelin is apparently the only monomeric protein without cysteine residues which occur in the precursor [12]. This adipokine was described in various tissues and organs such as the uterus, ovary, heart, lung, stomach and brain. The expression of apelin increases during adipocyte differentiation. Insulin, growth hormone (GH) or tumor necrosis factor (TNF-α) are among the agents regulating the production of apelin [13]. Apelin exerts some influence on the cardiovascular system, food intake, fluid homeostasis and energy metabolism. According to Castan-Laurell et al. [14], lean women exhibit plasma apelin levels in the range of 272 pg/ml, indicating a positive correlation between the plasma apelin level and body mass index (BMI).

2.3. Chemerin

Chemerin was first termed tazarotene-induced gene 2 protein (TIG2) and retinoic acid receptor responder protein 2 (RARRES2). It has been suggested to play a role in the metabolic syndrome. It is secreted as 143 residue prochemerin, which is largely expressed in the liver, WAT, skin, pancreas and kidneys. It is also the main form in the plasma. The level of active chemerin is negligible in basal conditions [15]. Various isoforms of chemerin have been detected in different tissues and fluids, including plasma, synovial fluid, muscles, liver

and ovary. Three isoforms have been identified, chemerinS, chemerinF and chemerinK and three G-protein coupled receptors have been detected, CMKLR1 (chemokine-like receptor1), GPR1 (G protein-coupled receptor 1) and CCRL2 (chemokine receptor like 2). CMKLR1 is coupled with the Gi/o proteins and inhibits the cyclic adenosine 3′,5′-monophosphate (cAMP) signaling pathway, promoting Ca^{2+} mobilization and extracellular signal–regulated kinases (ERK1/2) activation. The CMKLR1 sequence is closely related to GPR1 and activates the same pathways, while the role of CCRL2 remains unclear. Chemerin binding does not promote any signaling pathway and does not induce CCRL2 internalization [15]. Chemerin was reported to regulate adipogenesis, adipocytes differentiation, insulin secretion, inflammation and blood pressure. Obesity and type 2-diabetes are associated to high levels of chemerin [15].

2.4. Resistin

Resistin is a cysteine-rich, secretory protein, also known as adipocyte secreted factor (ADSF), belongs to the family of Found in Inflammatory Zone (FIZZ) proteins [16]. Resistin is produced by the adipocytes in mice whereas it is predominantly expressed in macrophages in humans. Human resistin is a 12.5 kDa cysteine-rich peptide with a mature sequence consisting of 108 amino acids, while the rat and mouse resistin has 114 amino acids. The comparison of the amino acid sequences of bovine resistin with that of humans, pigs, rats and mice showed 73, 80, 58 and 57% identity, respectively [17]. The resistin concentration in human plasma and follicular fluid ranges from 5 to 50 ng/ml [18], while in the follicular fluid in pigs it is around 0.323 ng/μg protein, depending on the stage of the estrous cycle [19]. Recent reports have suggested potential receptors for resistin, such as an isoform of decorin (DCN), mouse receptor tyrosine kinase-like orphan receptor 1 (ROR1), toll-like receptor 4 (TLR4) or adenylyl cyclase-associated protein 1 (CAP1) [5]. Furthermore, it is well known that resistin activates signaling pathways in different tissues like the phosphatidyl inositol 3′ kinase/ protein kinase B (Akt), mitogen-activated protein kinases (MAPK) (ERK1/2 and p38), signal transducer and activator of transcription 3 (Stat-3) and PPAR type gamma (PPARγ). Several studies have identified positive correlations between resistin levels and the pathogenesis of obesity, adipogenesis and insulin resistance [16].

2.5. Vaspin

Vaspin, a member of the serine protease inhibitor family, has been identified in the visceral adipose tissue of Otsuka Long-Evans Tokushima fatty rats at an age when body weight and hyperinsulinemia peaked [20]. This adipokine was initially observed in mature adipocytes from epididymal, retroperitoneal, mesenteric and subcutaneous abdominal WAT from 30-week-old Otsuka Long-Evans Tokushima fatty (OLETF) rats [20]. Literature data indicates the involvement of vaspin in the development of obesity, insulin resistance or pathogenesis of the body's inflammatory reactions [21]. Vaspin receptors remain unknown, but some data suggest that vaspin acts as a ligand for the cell-surface GRP78/voltage-dependent anion channel complex [22]. The vaspin gene was expressed in new born and adult bovines; in new born animals, vaspin is highly expressed in the heart, small intestine, skeletal muscle and fat, whereas in adults, it is expressed in the heart, liver, lungs and skeletal muscles. Vaspin increased the Akt phosphorylation protein and decreased the nuclear factor NF-κB level in pancreatic β cell and in cultured endothelial cells [23].

2.6. Visfatin

Visfatin was first discovered as a growth factor (PBEF) and then as a type II nicotinamide phosphoribosyltransferases (NAMPT). Visfatin structure showed a dimer organization separated by an active site. The visfatin gene is highly conserved among different species, such as mice, rats, humans and fish. The expression has been studied in many organisms and the porcine visfatin is ubiquitously expressed in tissues [24]. In a canine model, visfatin protein is expressed in various tissues like the liver, heart, brain, lungs and muscle [25]. In cows, mammary epithelial cells express visfatin [26]. In chickens, the full-length cDNA of visfatin gene has been cloned and sequenced. In the latter species, visfatin is highly expressed in muscles and it is involved in muscle growth [27]. Because of this characteristic, visfatin is also called myokine. No receptor has yet been identified for visfatin, but it is able to activate the insulin receptor. Besides muscle growth, visfatin is also involved in glucose and fatty acids metabolism and in reproductive functions. Moreover, in pancreatic β-cells, visfatin increases the glucose-stimulated insulin secretion.

3. Reproduction in domestic animals

The ovary, by producing steroid hormones, has a key role in female reproduction. It ensures follicles growth and the timely release of fertilizable oocytes essential for pregnancy, by directing feedback mechanisms on the hypothalamus and pituitary. Disruption of this finely controlled network can lead to many clinical syndromes including premature ovarian failure, ovarian hyperstimulation syndrome, ovulation defects, poor oocyte quality and cancer. Generally, in domestic animals, the oestrus cycle is composed of four phases: proestrus, estrus, metestrus and diestrus [28]. Each of these stages is a subdivision of the follicular and luteal phases of the cycle. For example, the follicular phase includes proestrus and estrus, while the luteal phase includes metestrus and diestrus [28]. Proestrus is characterized by a significant rise in estradiol (E2) produced by developing follicles. When E2 reaches a certain level, the female enters behavioral estrus and then ovulates. Following ovulation, cells of the follicles are transformed into corpus luteum (CL) cells during metestrus. Next, diestrus is characterized by a fully functional CL and high progesterone (P4) concentration [28].

3.1. Cows

The initiation of puberty is achieved when heifers reach 40 to 55% of their adult weight, which is strongly influenced by the nutritional level received during the prepubertal period [28]. At traditional high levels of energy intake, the onset of puberty may range from 7 to 10 months of age, 6 to 9 months earlier than for heifers of the same breed fed with a low energy nutritional intake [28]. Before the onset of puberty, the frequency of luteinizing hormone (LH) peaks increases leading to a brief P4 priming followed by the pubertal preovulatory surge of LH associated with behavioral estrus. The ovaries of heifers contain growing follicles that release steroidogenic hormones: P4 and E2 are regulated by the endocrine retro-control of the hypothalamus-pituitary-ovarian axis. In mature cows, waves of follicular oestrogens from granulosa cells (GC) activate gonadotropin releasing hormone (GnRH) and LH pulsation

that induce a positive feedback on E2 secretion until it reaches the preovulatory peak of LH and triggers ovulation. In cattle, the wave-like pattern of follicle development is very well characterized with most estrous cycles comprising two or three waves [28] (**Figure 2**). In the absence of fertilization, the endometrium secretes prostaglandins and CL regress and become atretic. Conversely, when fertilization occurs, CL cells continue to secrete P4 that stop the progression of a new estrous cycle [28]. In bovines, the gestation lasts 9 months. In breeding, farmers and researchers often resort to hormonal stimulation to synchronize ovulation and artificial insemination (AI). Over generations, the genetic selection and the high management for milk production have led to drastic negative energy balance and decline of fertility [28]. "Fertil+" cows had a significantly higher success rate at the first AI than "Fertil−" cows, without variations of ovarian dynamics. Traditionally, a decrease in the pregnancy rate at first service and the increase in the calving intervals and calving to first AI interval are associated with prolonged postpartum anestrus (the first ovulation is delayed), abnormal estrous cycles or follicular cyst formation [28].

3.2. Pigs

Pigs reach puberty close to the 7th month of postnatal life (**Table 1**); animals with higher fat mobilization reach sexual maturity faster and have more estrous cycles compared to animals with lower fat mobilization [29, 30]. The ovary begins to form at the 24-26th week of prenatal life, and the first ovarian follicles appear about 70 days after fertilization. Primary ovarian follicles comprise an oocyte surrounded by a single flattened layer of GC outlined by a basement membrane. Secondary follicles have a higher volume and number of GC and follicular

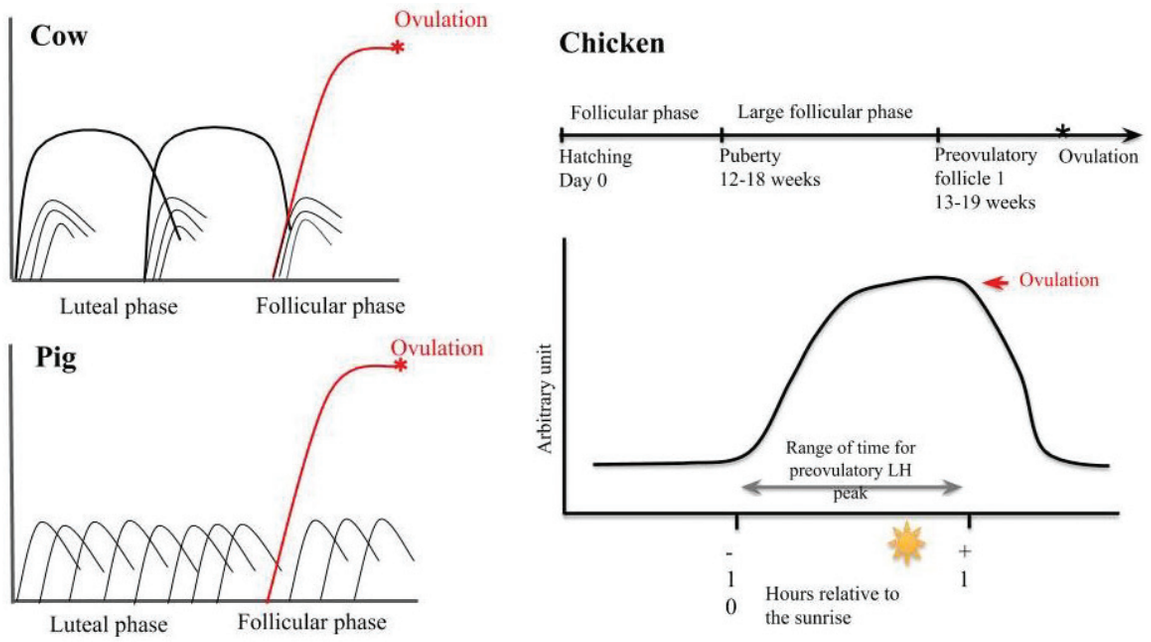

Figure 2. Schematic presentation of the pattern of follicle development during estrous cycles in cattle and pigs. In chickens, the pre-ovulatory release of LH can only be initiated between the beginning of the night and 1 hour after the sunrise. In addition, the ovulation is induced by a P4 peak under the control of LH.

Animal	Onset of puberty	Age at first Service	Estrous Cycle	Estrus	Gestation
Cows	1–2 yr	1–2 yr	21 d	18 h	282 d
Pigs	7 mo	8–10 mo	21 d	2 d	114 d
Chickens	12–18 wk	21 wk	24 h	no	21d

Table 1. Average ages or times for some reproductive parameters selected by species [28].

sheath is formed. Antral follicles formation depends on the follicular fluid accumulation, GC proliferation and theca cells (TC) differentiation. Preovulatory follicles are the final stage of the follicle before ovulation [29]. Swines do not show the wave-like pattern of follicle development that is so typical in ruminant species [28, 30]. While there is a coordinate development of follicles at the beginning of the luteal phase, there is continuous growth and atresia of ovarian cells during the rest of the luteal phase without evidence of dominant follicles or follicular waves (**Figure 3**) [29]. The outer wall of the ovarian follicle is the TC externa and the inner called TC interna. The TC externa implements an isolating function, while the TC interna function is secretory. The internal wall of the antral follicle is composed of GC, separated from the TC interna by the basement membrane. The last structure distinguished in the follicle is the oocyte, surrounded by the zona pellucida and corona radiata of granular cells [28]. The ovarian maturation is possible owing to the increasing number of follicle-stimulating

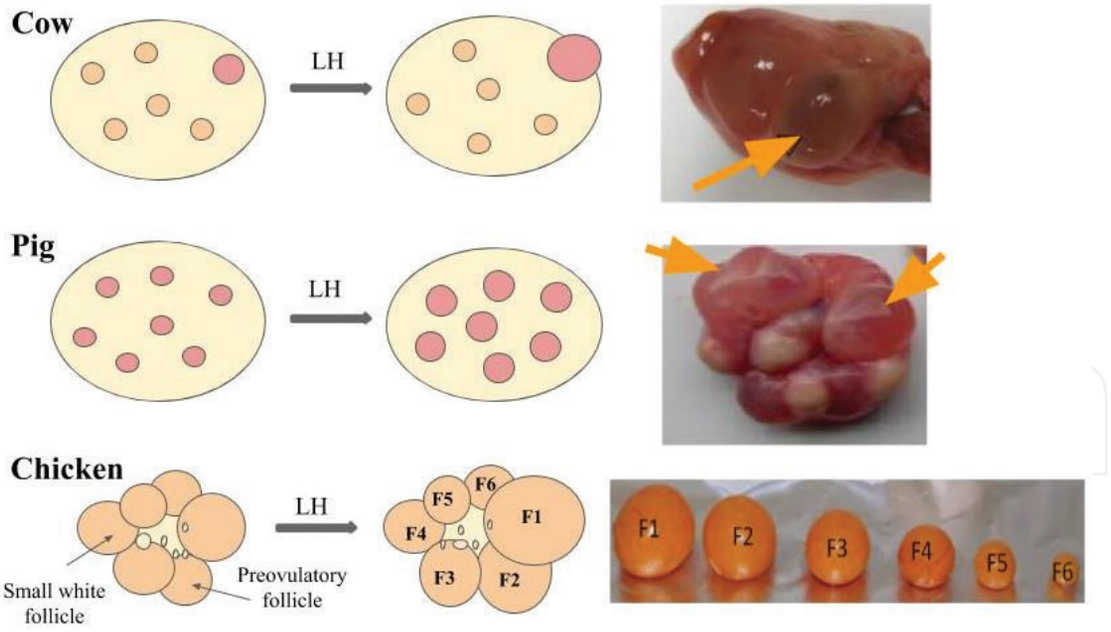

Figure 3. Ovarian follicles development and morphology. In cows, an early event in follicular selection is the acquisition of LH receptors in the GC of the presumptive dominant follicle. In pigs, multiple follicles acquire LH receptors in the GC at a less mature stage of follicular development; more preovulatory follicles are selected in pigs because more follicles have the capacity to survive during the transition from FSH to LH dependence. In reproductively active chickens, the ovarian cortex contains a hierarchy of follicles in all stages of development, from primordial follicles to large, yellow, yolk-filled pre-ovulatory follicles. AT gross inspection, the large follicles protrude from the surface of the ovary on short stalks while small follicles bulge from the surface.

hormone (FSH) receptors, which activates the aromatase complex involved in converting testosterone into E2. The secretion of E2, in turn, promotes the formation of the LH receptor in GC [29]. Pigs' estrous cycle lasts 21 days and is distinguished at 4 periods: proestrus, oestrus, metaestrus and diestrus. In the ovarian cycle two phases are distinguished: follicular (7 days), in which ovarian follicles are recruited and matured, and the luteal phase (14 days). The phases are separated by ovulation. The luteal phase starts in the first day after ovulation, and continues until day 15 of the estrous cycle. The early follicular phase starts on day 16–17 [29]. During the oestrus cycle different hormonal profiles are observed: E2 and inhibin gradually increase after luteolysis, and peak in the periovulatory period, provoking the LH surge. LH influences the final oocyte maturation, ovulation and CL formation. In the luteal phase, CL takes over the production of steroid hormones and secretes P4, which prepares the endometrium for embryo implantation and maintaining pregnancy for the first 12 weeks [28]. Porcine CL remains insensitive to prostaglandin F2α up to day 12 of the cycle; prostaglandin F2α degrades the luteal capillaries and reduces P4 production, leading to CL death. When there is no fertilization, the CL changes to atretic CL [28, 30]. Luteolysis begins on the 13th day of the cycle when the endometrium releases F2α prostaglandins, P4 synthesis is inhibited and new follicles grow. The percentage of fertilizations ending in pregnancy in pigs is high and reaches to 90%. Pregnancy lasts 112–114 days, producing a litter of 8–12. CL is essential for maintenance of pregnancy in pigs [30].

3.3. Chickens

Firstly, in birds, the sexual chromosomes are different from mammals. The female is the heterogametic with the chromosomes Z and W, while the male is homogametic with two Z chromosomes [31]. Then, in almost all species of birds, only the left ovary is functional. In hens, it develops after 16 weeks of age. It is organized in a single hierarchy consisting approximately of 6 preovulatory follicles. F1 is the largest preovulatory follicle and F6, the most recently selected from the cohort [31] (**Figure 3**). In domestic hens, sexual maturation occurs at about 5 months of age and is closely linked to the photoperiod. The follicular growth is associated with the formation of the TC interna layer. The preovulatory follicles rapidly grow and can reach more than 40 mm in diameter. The process of follicular selection is based on the ability of the FSH receptor to initiate cell signaling via cyclic adenosine monophosphate specifically in the GC [31]. Like mammal species, the preovulatory surge of LH is the first stimulus for the germinal vesicle breakdown and for ovulation. In avian species, the LH peak precedes ovulation by 4–6 hours. The largest preovulatory follicle produces a greater amount of P4 during the LH surge. This production is related to high levels of STAR in the GC of the F1 follicle [31]. Contrary to mammal species, there is no apparent increase in circulating FSH corresponding to the LH peak. Following the ovulation, the steroid production decreases and the regression of the postovulatory follicle is related to apoptosis, inducing the production of immune cells and cytokines. In birds, TC expresses aromatase and synthetize estrogens from androgens precursors, which are localized in TC. However, P4, pregnenolone and androgen precursors are produced almost exclusively within the TC interna, while GC produces P4 from cholesterol. These cells are able to convert P4 to testosterone but not to oestrogens [31]. Moreover, the hen oviduct is able to store sperm for a prolonged period in specialized sperm

storage tubules (SST), located in the utero-vaginal junction of the oviduct. Only the "normal" spermatozoa enter the SST indicating that some process of selection occurs [31].

4. Effect of adipokines on the ovarian function in domestic animals

Based on the available data, it is known that adipokines and their receptors are expressed in the ovarian cells and can modulate ovarian physiology, especially steroidogenesis, cell proliferation, apoptosis and/or oocyte maturation in domestic animals like pigs, cows, chickens, goats, ewes and turkeys (**Table 2**).

4.1. Adiponectin

In cows, serum adiponectin decreases from 21 days before calving, reaches a nadir at calving, before rapidly decreasing upon advancement of lactation [32]. During the bovine oestrus cycle, serum adiponectin levels range between 27 and 32 μg/ml and were 1.6-fold lower in

Adipokines	Ovarian function in domestic animals		
	Cows	**Pigs**	**Chickens**
Adiponectin	↓ A4, LHR, CYP11A1, CYP17α1; ↑ IGF1-induced and basal proliferation; ↓ insulin-induced P4 and E2	Follicles: ↑ expression of genes associated with periovulatory remodeling; ↑E2; CL: ↓ P4 cells	↑ IGF1-induced P4 ↓ LH and FSH-induced P4
Apelin	↑ IGF1-induced P4 and cell proliferation; ↓ IGF1-induced oocyte maturation and P4 from cumulus cells through MAPK ERK1/2	Follicles: ↑ basal P4, E2; ↓ IGF1-, FSH-induced P4, E2; ↑ proliferation; CL: ↑ P4 and 3βHSD	nd
Chemerin	↓ P4 and E2 at basal and in response to IGF1 or FSH through CMKLR1; ↓ StAR, CYP19 and MAPK-ERK1/2	nd	nd
Resistin	↑ FSH and IGF1-induced E2; no effect on IGF1- or insulin-induced P4 and A4 production by Tc or P4 production by Gc of large follicles; ↑ IGF1-induced P4 and E2 in small follicles;	↑ basal P4, T and A4; ↓ gonadotropin- and IGF1-induced P4, T, A4 and E2 by inhibition of 3βHSD, 17βHSD and CYP19A1; ↓ apoptosis;	nd
Visfatin	↑ basal and IGF1-induced steroid secretion; ↑ StAR, 3βHSD, IGF1-R, MAPK ERK1/2	nd	↓ IGF1-induced P4

CL, Corpus luteum; IGF1, Insulin-like factor 1; FSH, Follicles stimulation hormone; P4, Progesterone; A4, Androstenedione; E2, Estradiol; IGF1, Insulin-like growth factor 1; CL, Corpus luteum; Tc, Theca cells; ↑, increased; ↓, decreased; nd, no data.

Table 2. Effect of adipokines on the ovarian physiology in domestic animals.

follicular fluid, but the concentrations in the serum and follicular fluid were not correlated [33]. The circulating levels of adiponectin were decreased when providing low energy diet to the cows, and were related to a delayed and abnormal luteal activity in lactating cows [34]. *In vitro*, adiponectin (3 μg/ml) decreases the production of androstenedione (A4) by TC in cows by reducing the expression of LH receptors and CYP11A1 (cytochrome P450, family 11, subfamily a, polypeptide 1) and CYP17α1 (cytochrome P450, family 17, subfamily a, polypeptide 1) enzymes mediated by both receptors AdipoR1 and AdipoR2. With a higher dose (10 μg/ml), adiponectin increases insulin like growth factor 1 (IGF1)-induced GC and basal TC proliferation [33]. Additionally, adiponectin decreases insulin-induced secretions of P4 and E2 by GC but has no effect on the nuclear maturation of oocytes or early embryo development [5].

In pigs, adiponectin and its receptors expressions are observed in the ovary and a decrease of the 250 kDa adiponectin isoform has been reported in both follicular fluid and serum [35]. Adiponectin system is also expressed in the endometrium, myometrium and conceptuses and is dependent on the stage of the pregnancy. The highest level of adiponectin is observed on days 15 to 16 of the pregnancy and on days 10 to 11 of the cycle on the endometrium. The highest expression of AdipoR1 and AdipoR2 is detected on days 10 to 11 in the endometrium and on days 12 to 13 in the myometrium [36], around the period of the maternal recognition of pregnancy. The *in vitro* studies on porcine uterine tissues showed that adiponectin affects the gene expression of key enzymes involved in the steroid synthesis (StAR, CYP11A1 and 3β-HSD), and influences P4 and A4 secretion and prostaglandin synthesis pathway in the porcine uterus [37]. Using GC collected from medium-sized (3–5 mm) follicles of prepubertal gilts ovaries, Ledoux et al. [38] showed that adiponectin is present in the porcine follicular fluid at concentrations similar to those found in serum. These authors demonstrated that adiponectin at physiologically relevant levels (10–25 μg/ml), induces the expression of genes like cyclooxygenase-2, prostaglandin E synthase, and vascular endothelial growth factor genes, which are associated with periovulatory remodeling of the ovarian follicle [38]. The expression of adiponectin is significantly higher in porcine CL during the luteal phase compared with TC isolated on days 17–19 of the cycle [39]. Adiponectin at doses between 1 and 10 μg/ml significantly decreased P4 secretion by CL cells *in vitro*, and increased E2 secretion by GC, but had no effect on T secretion by TC [39].

In chickens, the adiponectin gene was found in the ovary, and it was 10- to 30-fold higher expressed in TC than in GC from each of the follicles (F1-F4) [40]. For the receptors, the AdipoR1 mRNA level is two-fold lower in TC than GC, while the AdipoR2 expression remained stable in both ovarian cells and during follicular development. Adiponectin exerts an autocrine or paracrine effect on ovarian steroidogenesis. In F2 and F3/4 follicles, adiponectin increased IGF1-induced P4 production. In F3/4 follicles, it decreases P4 production in response to LH and FSH [40].

In turkeys, the adiponectin plasma profile is significantly lower at the end compared to the beginning of the laying period. Furthermore, the hexameric form is reduced by three-fold at the end in comparison to the start of this period [41].

4.2. Apelin

In bovine species, it has been shown that apelin and apelin receptor (APJ) mRNAs are expressed in the bovine follicle and CL [42]. In the CL, apelin expression increases during the

early luteal stages and decreases at the end of the luteal phase and during CL regression [42]. Apelin expression is only higher during pregnancy [42]. Thus, the apelin/APJ system could be involved in vascular establishment, maturation and maintenance of the CL during the estrous cycle. In TC, the expression of apelin is increased by E2 (5–180 ng/ml) and LH (100 ng/ml), while the expression of APJ is only increased by LH. In GC, the expression of APJ is increased by P4 (10 ng/ml) and by FSH (100 ng/ml) [43]. In mature bovine follicles, the apelin/APJ is thought to play a crucial role during follicle selection and dominance. During an *in vitro* study, apelin (10^{-9} M) increases IGF1-induced P4 secretion and cell proliferation in bovine luteinizing GC, whereas it inhibits IGF1-induced oocyte maturation and P4 secretion from cumulus cells through the regulation of extracellular signal-regulated kinases MAPK ERK1/2 phosphorylation [42–44].

In porcine, apelin concentration in the follicular fluid and its expression increases with ovarian follicular growth. Immunohistochemistry revealed the positive higher staining for apelin in membranes of GC, than TC [45]. Apelin was found to increase secretion and 3βHSD and CYP19 expression in co-culture of GC and TC as well as cell proliferation, while decrease IGF1- and FSH-induced steroidogenesis [45]. As a molecular mechanism of these observation, authors showed that AMPKα was involved in the action of apelin on P4 production but MAPK/ERK, AMPKα and Akt/PI3 mediated the proliferative effect of apelin [45]. In an *in vitro* experiment involving CL cells, apelin also increased P4 secretion by the activity of 3βHSD [46]. Expression of mRNA apelin in CL were similar in the early and middle CL, and less in late CL. Apelin has been shown in the cytoplasm of both, small and large luteal cells by immunohistochemistry [46].

4.3. Chemerin

In bovine species, chemerin reduces *in vitro* P4 and E2 secretion at basal levels, in response to IGF1 or FSH through its main receptor, CMKLR1 [47]. Chemerin also reduces cholesterol content, the protein quantity of the cholesterol carrier StAR, the protein CYP19 and the level of phosphorylation of the MAPK-ERK1/2 in the presence or absence of IGF1 and FSH [47]. In the bovine cumulus-oocyte complex, chemerin arrests the majority of oocytes at the GV stage *in vitro* which is associated with a reduction in P4 secretion by the cumulus cells and the phosphorylation of the MAPK-ERK1/2 signaling pathway in the oocyte and cumulus cells [47].

In chickens, chemerin and its receptors are higher in TC than in GC in both preovulatory follicle 1, follicle 3 and 4 [48]. Chemerin is negatively correlated with the rate of hatchability. In contrast, the weight of the preovulatory follicle F1 is positively correlated with the chemerin expression in GC. Finally, the P4 production in GC is negatively correlated with the chemerin expression in TC. In hens, the diet has a significant impact on the reproductive function [48]. Thus, restricted fed hens expressed lower chemerin mRNA expression in TC from preovulatory follicles 1 and 3 than ad libitum fed hens. A fish oil supplementation increases the mRNA level of CMKLR1 in TC of preovulatory follicle F1 but decreases it in TC of preovulatory follicle F3 [48]. The chemerin gene sequence is similar at 81% between chickens and turkeys. In turkeys, chemerin concentration in plasma decreases at the end of the laying and is negatively correlated with phospholipids, triglycerides and cholesterol levels during this period [41]. The role of chemerin in the different mechanisms of the reproduction remains to be considered.

4.4. Resistin

Resistin is widely expressed in differently sized bovine follicles (small <6 mm and large >6 mm), where it was demonstrated in oocytes, cumulus, TC and GC, as well as in the CL [49]. In cattle, resistin at 30 ng/ml weakly stimulated FSH plus IGF1-induced E2 production but had no effect on IGF1- or insulin-induced P4 or A4 production by TC or P4 production by GC of large follicles [50]. However, in GC from small follicles, resistin attenuated the stimulatory effect of IGF1 on P4 and E2 secretion. Moreover, it has been documented that, resistin stimulated Akt and p38-MAPK phosphorylation in bovine and rat GC, ERK1/2-MAPK phosphorylation in rats and had the opposite effect on the AMPK pathway [49].

In pig ovaries, resistin levels and expression varies with the stage of cycle. Differences in the resistin expression and concentration in follicular fluid collected from small, medium, and large follicles have also been reported [19]. Interestingly, in contrast to prepubertal animals, resistin expression and concentration in adult estrous cycling pigs was independent of follicular size and/or development [5]. Moreover, several factors can influence ovarian resistin expression, which has shown to increase with gonadotropin and steroid hormones and decrease with IGF1 and rosiglitazone (a PPARγ-selective agonist) [5]. Resistin affected the ovarian steroidogenesis, decreasing gonadotropin- and IGF1-induced steroid hormone secretion by the inhibition of 3βHSD, 17βHSD and CYP19A1 protein expression [5]. In ovarian follicles resistin by direct effects on both death receptor- and mitochondria-mediated apoptosis protein, was described as an anti-apoptotic factor [5]. It has been proposed the activation of several signal transduction pathways, such MAPK/ERK1/2, Janus-activated kinases (JAK)/STAT and phosphatidylinositol 3-kinase (PI3K) as a molecular mechanism of resistin action on cell survival [5]. These results suggest the involvement of resistin in ovarian apoptosis regulation and could regulate follicular development or atresia.

4.5. Visfatin

In cumulus cells from "Fertil−" cows, visfatin mRNA expression was lower compared to "Fertil+" cows, especially after *in vitro* maturation [51]. During *in vitro* experiments, visfatin improves basal and IGF1-induced steroidogenesis, probably through increasing the protein level of StAR, the 3βHSD activity and the phosphorylation levels of IGF1-receptor and MAPK ERK1/2 [52].

In chickens, visfatin mRNA is higher in GC than in TC, and it regulates steroidogenesis in ovarian cells. During the folliculogenesis, the expression in TC decreases whereas it remains stable in GC. It inhibits IGF1-induced P4 production in GC. Moreover, the plasma level is significantly lower in adult hens than in juveniles [53]. In turkeys, visfatin mRNA level is higher in TC than in GC in follicles F1, F3 and F4, like chemerin and adiponectin expression. Moreover, visfatin concentrations in plasma decrease and are negatively correlated to plasma glucose during the laying period [41].

4.6. Vaspin

Vaspin expression and roles on female reproduction remain unknown. In porcine ovarian follicles, our preliminary results evidenced mRNA and protein expression of vaspin, whose

expression was decreased during ovarian follicle development. We also observed that protein expression of vaspin was lower in large follicles of low-fat mobilization pigs (*Large White*) than in high fat mobilization pigs (*Meishan*), but this data should be confirmed.

5. Conclusion

Taken together, this chapter summarizes the expression and direct role of different adipokines in ovarian follicle cells. These observations clearly documented that adiponectin, apelin, chemerin, resistin, visfatin and vaspin are expressed on mRNA and protein level in the ovarian GC, TC and CL, suggesting that the ovary is a target organ for adipokines production and secretion. It is interesting that several *in vitro* experiments have documented that these peptides can regulate the ovarian physiology such as the steroid hormone production cell proliferation, apoptosis and oocyte maturation in different domestic animals like pigs, cows, chickens or turkeys, and should be considered a newly identified regulator of female reproduction.

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Conflicts of interest

The authors declare no conflicts of interest.

Abbreviations

WAT	white adipose tissue
HMW	high-molecular weight
AdipoR1	adiponectin receptor 1
AdipoR2	adiponectin receptor 2
PPAR	proliferator-activated receptor
APJ	apelin receptor
GH	growth hormone
TNF-α	tumor necrosis factor

BMI	body mass index
TIG2	termed tazarotene-induced gene 2 protein
RARRES	retinoic acid receptor responder protein 2
CMKLR1	chemokine-like receptor 1
GPR1	G protein-coupled receptor 1
CCRL2	chemokine receptor like 2
cAMP	cyclic adenosine 3′,5′-monophosphate
ERK1/2	extracellular signal–regulated kinases
ADSF	adipocyte secreted factor
FIZZ	inflammatory zones
DCN	decorin
ROR1	tyrosine kinase-like orphan receptor 1
TLR4	toll-like receptor 4
CAP1	adenylyl cyclase-associated protein 1
Akt	protein kinase B
MAPK	mitogen-activated protein kinases
Stat-3	signal transducer and activator of transcription 3
OLETF	Otsuka Long-Evans Tokushima fatty
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
PBEF	pre b cells enhancing factor
NAMPT	type II nicotinamide phosphoribosyltransferases
E2	estradiol
CL	corpus luteum
P4	progesteron
LH	luteinizing hormone
GC	granulosa cells
GnRH	gonadotropin releasing hormone
AI	artificial insemination
FSH	follicle-stimulating hormone
TC	theca cells

StAR	steroidogenic acute regulatory protein
SST	sperm storage tubules
A4	androstenedione
LHR	the luteinizing hormone receptor
CYP11A1	cytochrome P450, family 11, subfamily a, polypeptide 1
CYP17α1	cytochrome P450, family 17, subfamily a, polypeptide 1
IGF-I	insulin-like growth factor-1
3βHSD	3β-Hydroxysteroid dehydrogenase/Δ^{5-4} isomerase
17βHSD	17β-Hydroxysteroid dehydrogenases
IGFi-R	insulin-like growth factor-1 receptor
AMPKα	5′AMP-activated protein kinase
GV	germinal vesicle
PI3K	phosphatidylinositol 3-kinase
JAK	Janus-activated kinases

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